

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A trap vector comprising a *loxP* sequence and a ~~mutant *loxP*~~ *lox71* sequence,

wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence, and inverted repeat sequence 2; and

~~wherein the mutant *loxP* sequence comprises a sequence in which a part of said inverted repeat sequence 1 of *loxP* is mutated such that recombination of the mutant *loxP* occurs more efficiently than the reverse reaction as compared to wild-type *loxP*~~ wherein the *lox71* sequence comprises a nucleotide sequence shown in SEQ ID NO:15; and

wherein the trap vector is suitable for use in mammalian cells.

2-3. (Cancelled)

4. (Currently Amended) A trap vector comprising a *loxP* sequence and a ~~mutant *loxP*~~ *lox66* sequence, wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence, and inverted repeat sequence 2; and,

~~the mutant *loxP* sequence comprises a sequence in which a part of said inverted repeat sequence 2 of *loxP* is mutated such that recombination of the mutant *loxP* occurs more efficiently than the reverse reaction as compared to wild-type *loxP*~~ wherein the *lox66* sequence comprises a nucleotide sequence shown in SEQ ID NO:16; and

wherein the trap vector is suitable for use in mammalian cells.

5-6. (Cancelled)

7. (Previously presented) A trap vector selected from the group consisting of the following (a) to (i):

- (a) SP-SA-*lox71*-IRES-M-*loxP*-PV-SP;
- (b) SP-*lox71*-IRES-M-*loxP*-PV-SP;
- (c) SA-*lox71*-IRES-M-*loxP*-pA-PV-SP;
- (d) SA-*lox71*-IRES-M-*loxP*-*puro*-pA-PV-SP;
- (e) *lox71*-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (f) *lox71*-IRES-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (g) (*lox71*-integrated SA)-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (h) (*lox71*-integrated SA)-IRES-M-*loxP*-pA-*lox2272*-PV-*lox511*; and
- (i) (*lox71*-integrated SA)-M-*loxP*-pA-*lox2272*-promoter-M-*lox511*-SD;

wherein SP represents any sequence; SA represents a splice acceptor; SD represents a splice donor; IRES represents an internal ribosomal entry site; M represents a marker gene; *puro* represents puromycin resistance gene; pA represents a poly(A) sequence; and PV represents a plasmid vector.

8. (Original) The trap vector of claim 7, wherein the plasmid vector is any one selected from the group consisting of pBR, pUC, pSP and pGEM.

9. (Previously Presented) A vector generated from recombination between:

(a) a trap vector comprising a *loxP* sequence and a mutant *loxP* sequence, wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence, and inverted repeat sequence 2; wherein the mutant *loxP* sequence comprises a sequence of which a part of said inverted repeat sequence 1 of *loxP* is mutated; and

(b) a trap vector comprising a *loxP* sequence and a mutant *loxP* sequence, wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence and inverted repeat sequence 2; wherein the mutant *loxP* sequence comprises a sequence of which a part of said inverted repeat sequence 2 of *loxP* is mutated,

wherein recombination of the mutant *loxP* occurs more efficiently than the reverse reaction as compared to wild-type *loxP*.

10. (Previously presented) The vector of claim 9, wherein said vector does not undergo recombination with another *loxP*.

11. (Currently Amended) A method of gene trapping, comprising the steps of:
introducing the trap vector of any one of claims ~~1 to 8~~ 1, 4, 7, 8 or 21 into embryonic stem cells;
culturing the embryonic stem cells;
selecting those cells which exhibit a pattern of single copy integration of the trap vector; and
isolating the trapped gene.

12. (Currently Amended) Embryonic stem cells into which the trap vector of any one of claims ~~1 to 8~~ 1, 4, 7, 8 or 21 is introduced.

13-18. (Canceled)

19. (Currently Amended) A method of gene trapping, said method comprising the steps of:

introducing into embryonic stem cells:

(a) a trap vector comprising a *loxP* sequence, a marker gene and a mutant *loxP* sequence, wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence, and inverted repeat sequence 2; wherein the mutant *loxP* sequence comprises a sequence of which a part said inverted repeat sequence 1 of *loxP* is mutated; or

(b) a trap vector comprising a *loxP* sequence, a marker gene and a mutant *loxP* sequence, wherein the *loxP* sequence comprises in sequential order inverted repeat sequence 1, a spacer sequence and inverted repeat sequence 2; wherein the mutant *loxP* sequence comprises a sequence of which a part of said inverted repeat sequence 2 of *loxP* is mutated,

wherein recombination of the mutant *loxP* occurs more efficiently than the reverse reaction as compared to wild-type *loxP*; and

wherein both trap vectors (a) and (b) further comprise at least one splice acceptor site and at least one internal ribosomal entry site;

culturing the embryonic stem cells;
selecting those cells which exhibit a pattern of single copy integration of the trap vector; and
isolating the trapped gene.

20. (Previously Presented) The method according to claim 19, wherein the trap vector further comprises pA and PV, wherein pA is located downstream of the marker gene.

21. (New) A trap vector selected from the group consisting of the following (a) to (i):

- (a) SP-SA-*lox66*-IRES-M-*loxP*-PV-SP;
- (b) SP-*lox66*-IRES-M-*loxP*-PV-SP;
- (c) SA-*lox66*-IRES-M-*loxP*-pA-PV-SP;
- (d) SA-*lox66*-IRES-M-*loxP-puro*-pA-PV-SP;
- (e) *lox66*-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (f) *lox66*-IRES-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (g) (*lox66*-integrated SA)-M-*loxP*-pA-*lox2272*-PV-*lox511*;
- (h) (*lox66*-integrated SA)-IRES-M-*loxP*-pA-*lox2272*-PV-*lox511*; and
- (i) (*lox66*-integrated SA)-M-*loxP*-pA-*lox2272*-promoter-M-*lox511*-SD;

wherein SP represents any sequence; SA represents a splice acceptor; SD

represents a splice donor; IRES represents an internal ribosomal
entry site; M represents a marker gene; *puro* represents puromycin
resistance gene; pA represents a poly(A) sequence; and PV
represents a plasmid vector.